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Differential Requirements for Baculovirus Late Expression Factor Genes in Two Cell Lines

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A plasmid library of 18 late expression factor (LEF) genes (LEF library) from the baculovirus *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV) supports transient expression from a late viral promoter in the SF-21 cell line, derived from *Spodoptera frugiperda*. We found, however, that this LEF library was unable to support expression from the same promoter in the TN-368 cell line, derived from *Trichoplusia ni*, which is also permissive for Ac*M*NPV replication. To identify the additional factor(s) required for expression in TN-368 cells, we cotransfected the LEF library with clones representing portions of the Ac*M*NPV genome not represented in the LEF library. A single additional gene was identified; this gene corresponded to ORF70 of the complete Ac*M*NPV sequence and potentially encodes a 34-kDa cysteine-rich polypeptide. Because of its differential effect on late gene expression in the two cell lines, we renamed ORF70 *hcf-1* (for host cell-specific factor 1). *hcf-1* was involved in expression from reporter plasmids under late and very late but not early promoter control, indicating that it was also a LEF gene. Plasmid DNA replication assays indicated that HCF-1 was involved in virus origin-specific DNA replication in TN-368 cells. Three LEF genes, *ie-2*, *lef-7*, and *p35*, required for optimal virus origin-specific plasmid DNA replication or stability in SF-21 cells had little or no influence in TN-368 cells. Thus, as determined by transient-expression assays, cell line-specific and potentially host-specific factors are required for origin-specific DNA replication or stability.

The genome of *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV) consists of a double-stranded circular DNA molecule of 133,894 bp which can potentially encode up to 154 genes (1). Ac*M*NPV gene expression is coordinately regulated and appears to be primarily controlled at the level of transcription (for reviews, see references 3 and 45). Baculovirus early genes are apparently transcribed by host RNA polymerase II (15, 25, 26) and include gene products that are transcriptional regulators (5, 6, 19, 28, 33, 65) and factors thought to function in viral DNA replication (32, 44, 62). Studies with inhibitors of DNA synthesis (56) and with a temperature-sensitive Ac*M*NPV mutant defective in DNA synthesis (13, 16) demonstrate that expression of late and very late baculovirus genes is dependent on viral DNA replication. The switch from early to late gene expression is coincident with the appearance of an alpha-amanitin-resistant RNA polymerase activity (14, 18, 26, 64).

Eighteen genes which contribute to the expression of a reporter gene under late viral promoter control in transient-expression assays have been identified (31, 34, 41, 47–51, 60, 61) and have been termed late expression factor genes (LEFs). These 18 LEFs together represent all the viral genes necessary to support substantial expression from a late viral promoter in transient-expression assays in SF-21 cells (35, 60). A subset of these LEFs, *ie-1*, *ie-2*, *lef-1*, *lef-2*, *lef-3*, *lef-7*, *p143*, *dnapol*, and *p35*, are required for virus origin-specific plasmid DNA replication (27, 35), indicating the dependence of late gene expression on DNA replication even in a transient-expression assay system. The roles of the remaining LEFs are currently unknown, but they appear to function in late gene expression at the level of transcription (35). The products of *lef-8* and *lef-9* contain conserved RNA polymerase motifs (34, 51), suggesting that at least some of these LEFs may be components of a novel RNA polymerase activity induced late in infection.

The roles of the some of the replication-related LEFs are clearer. The gene products of *ie-1* and *ie-2* are transcriptional regulators (5, 6, 21, 28, 33, 41, 42, 47, 55, 65) and presumably are required for the expression of all of the other LEFs. The presence of a single-stranded DNA-binding motif within IE-1 (27) and its ability to bind to homologous repeat regions (19) suggest the possibility of an additional, direct role for IE-1 in DNA replication. DNAPOL and P143 contain amino acid motifs which are conserved among DNA polymerases and DNA helicases, respectively (32, 62), while LEF-7 has homology with the herpesvirus UL29 family of single-stranded DNA-binding proteins (35). Interestingly, DNAPOL has an auxiliary role in virus origin-specific plasmid DNA replication, suggesting that a cellular DNA polymerase can substitute for it, at least in transient-replication assays (35). P35 is required to inhibit apoptosis during Ac*M*NPV infection of *Spodoptera frugiperda* cells (7, 9, 23) and also has antiapoptotic activity in a number of diverse organisms (22, 53, 57). The role of *p35* in the plasmid replication assay is probably to maintain plasmid DNA stability by blocking apoptosis.

Two of these genes, *p143* and *p35*, have also been shown to be host range determinants. Recombinants of Ac*M*NPV which are able to productively infect *Bombyx mori* cells have been isolated following recombination of Ac*M*NPV with a small fragment of the *B. mori M*NPV *p143* gene (11, 37). How this region confers replicative competency on Ac*M*NPV in *B. mori* cells is unknown. Virus replication is significantly reduced in *S. frugiperda* cells and larvae but not in *Trichoplusia ni* cells and larvae infected with Ac*M*NPV mutants defective in *p35* (7, 8, 23), indicating that *p35* is a host range determinant.

Ac*M*NPV is able to enter a number of nonpermissive insect cells and express a reporter gene (4, 39, 40), but expression is dependent on the promoter class. Expression from early viral

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or cellular promoters transcribed by host RNA polymerase II is relatively high, but expression from late and very late promoters is low compared with that in permissive cells (39). It was proposed that the block to productive Ac*M*NPV infection in refractive cell lines is limited by factors which influence viral DNA replication and/or late gene expression (40).

Identification of the genes that allow Ac*M*NPV to successfully replicate in different cell lines will provide insight into the nature of the molecular mechanisms which govern host range and contribute to the assessment of recombinant baculovirus safety to nontarget organisms. In an effort to understand what viral factors influence viral DNA replication and late gene expression in Ac*M*NPV-infected cells, we have used a transient assay system in which the ability of the LEF library to support expression from a reporter plasmid under late promoter control was examined in the TN-368 cell line, derived from *T. ni*. Unlike in SF-21 cells, very little reporter gene activity was observed with the LEF library, indicating that an additional factor(s) was required for optimal reporter gene expression. This factor was identified as ORF70 (1). Transient-replication assays demonstrated that ORF70 was involved in reporter plasmid DNA replication or stability. We also found that three factors required for maximal expression in SF-21 cells had little or no influence on late gene expression or origin-specific DNA replication in TN-368 cells.

MATERIALS AND METHODS

Cells. *S. frugiperda* IPBL-SF-21 (SF-21) cells (63) and *T. ni* TN-368 cells (24) were cultured at 27°C in TC-100 medium (GIBCO BRL, Gaithersburg, Md.) supplemented with 10% fetal bovine serum and 0.26% tryptose broth (45).

Reporter plasmids and plasmid constructs. The reporter plasmids pETCAThr5, pCAPCAT, and phcwt have been described previously (47, 54, 59). These constructs contain the chloramphenicol acetyltransferase gene (*cat*) under the control of the early *etl* gene, the late *vp39* capsid gene, and the very late polyhedrin (*polh*) gene promoters, respectively. These reporter plasmids also contain *hr5* sequences.

The plasmids in the LEF library, which have been previously described, are pBCNE (*lef-1*) (48), p630 (*lef-2*), pIE1/HC (*ie-1*), and pPstN (*ie-2*) (47); pSDEM2 (*lef-3*) (31); p86D5B (*lef-4*) and pORF6 (*lef-5*) (49); pUCp143 (*p143*) (35); pAcIAP-Ps/NsiI (*lef-6*) (50); pBSXBglII (*lef-7*) (41); pRI-M (*lef-8*) (51); pPstHISB2.35 (*lef-9*), pPstHIME0.5 (*lef-10*), and pDNAp (*dnapol*) (34); and pH$_3$R (*lef-11*), pNspAfI (*39K*), p47 (*p47*), and pRS (*p35*) (60). Plasmids pXma17, containing the region of Ac*M*NPV from map units (m.u.) 39.48 to 49.94, and its subclones pXA-7, pXABB4.5, pXK2.1, pXBS2.1, pXAEBS, and pXABgE have been described (38).

Plasmid pXASstII1.5 was constructed by cloning a 1.5-kb *Sst*II fragment from pXABgE into the *Sst*II site of pBluescript KS$^+$ (Stratagene, La Jolla, Calif.). During this cloning, it was discovered that the original subclone of pXma17, pXA-7, previously thought to contain a *Bgl*II-*Sma*I fragment (43.96 to 49.94 m.u.), had sustained a 1.2-kb deletion at the *Bgl*II site during cloning. Thus, all previously reported subclones (38) derived from pXA-7 and including the *Bgl*II site at 43.96 m.u. actually begin at 44.68 m.u. (see Fig. 2A); this difference does not affect the conclusions of that study. Another subclone, pXABgE3.6, containing the entire region from 43.96 to 46.63 m.u., was constructed by digesting pXma17 with *Bgl*II and *Eco*RV and inserting a 3.6-kb fragment into the *Bam*HI and *Eco*RV sites in pBluescript KS$^+$. pXAMlu1.4, containing ORF70 and portions of ORF69 and ORF71, was constructed by digesting pXABgE3.6 with *Mlu*I and blunt-ending the products with the large fragment of DNA polymerase I (Klenow). A 1.4-kb fragment was gel isolated and cloned into the *Sma*I site of pBluescript KS$^+$. pXAMlu1.4fs was constructed by digesting pXAMlu1.4 with *Sst*I, blunt-ending it with T4 DNA polymerase, and religating it. The resulting plasmid, pXAMlu1.4fs, contains a frameshift mutation at the *Sst*I site at 45.15 m.u. that results in the premature termination of ORF70 synthesis after 112 amino acid residues. Sequence analysis was used to confirm that a frameshift mutation was generated at the *Sst*I site. Plasmid pXAIAP2 was constructed by cloning a 1.9-kb *Sst*I-*Eco*RV fragment from pXABgE3.6 into the corresponding sites in pBluescript KS$^+$. This plasmid contains ORF71 (a homolog of *iap*) (1), ORF72, and ORF73, as well as portions of ORF70 and ORF74.

The clones of the overlapping Ac*M*NPV library used in these experiments have been described previously (47).

Transfections and transient-expression assays. SF-21 (2.0×10^6 cells per plate) or TN-368 (1.8×10^6 cells per plate) cells were transfected with 2 μg of a reporter plasmid and 1 μg of each LEF plasmid by using Lipofectin reagent (GIBCO BRL). In experiments involving the Ac*M*NPV clone library, approximately 0.5 μg of each clone was used. Cell lysates were prepared at 24, 48, or 72 h post-transfection for transfections involving pETCAThr5, pCAPCAT, and phcwt, respectively. For purposes of quantitation, CAT assays (17) were performed with 1/100 of the cell lysates. Quantitations of CAT assays were done directly on the thin-layer chromatography plates with a PhosphorImager 4000 (Molecular Dynamics, Sunnyvale, Calif.).

DNA replication assays. Transfections of TN-368 cells were performed as detailed above except that pCAPCAT was used as the reporter plasmid in all transfections. Total intracellular DNA was isolated from cells at 96 h posttransfection. The procedures for DNA isolation and *Dpn*I analysis were performed as previously described (35). Briefly, the presence or absence of pCAPCAT plasmid replication was identified by digesting 1/10 of the DNA with *Bgl*II (40 U) and *Dpn*I (40 U) followed by agarose gel electrophoresis and transfer of the DNA onto a Zeta Probe nylon membrane (Bio-Rad, Richmond, Calif.). *Dpn*I-resistant fragments corresponding to linearized pCAPCAT plasmid (7.2 kb) that had replicated were visualized by hybridization of the blot with radiolabeled pCAPCAT DNA. The relative levels of pCAPCAT DNA replication were quantitated with the Molecular Dynamics PhosphorImager 4000.

RESULTS

Additional gene located between 44.6 and 50.3 m.u. is involved in late gene expression in TN-368 cells. A LEF library consisting of 18 plasmids is sufficient to support late gene expression to levels comparable to that observed for the complete overlapping Ac*M*NPV clone library in SF-21 cells (60). Removal of any individual LEF plasmid from this library substantially reduces late gene expression 10- to 100-fold (35). In order to determine whether these 18 LEFs were also sufficient to support pCAPCAT expression in a different cell line permissive for Ac*M*NPV infection, the LEF library was tested in TN-368 cells. Similar levels of CAT were observed in SF-21 and TN-368 cells transfected with the entire overlapping Ac*M*NPV clone library (Fig. 1B, compare lanes 2 and 5); however, whereas CAT levels were comparable between the clone library and the LEF library in SF-21 cells (Fig. 1B, compare lanes 2 and 3), the levels of CAT were reduced about 20-fold in TN-368 cells in the presence of the LEF library relative to the levels with the Ac*M*NPV clone library (Fig. 1B, compare lanes 5 and 6). This result suggested that the Ac*M*NPV clone library supplied one or more genes, in addition to the 18 LEF genes, that were necessary for expression of pCAPCAT in TN-368 cells.

To identify regions of Ac*M*NPV containing these genes, individual clones from the Ac*M*NPV clone library were cotransfected with the LEF library to determine their effects on pCAPCAT expression in TN-368 cells (Fig. 1B, lanes 7 to 18). Two clones, PstH5 and HC9, which overlap in the region between 44.6 and 50.3 m.u., stimulated CAT levels to a level similar to that observed with the complete Ac*M*NPV clone library (Fig. 1B, compare lanes 14 and 15 with lane 5). No significant increase in CAT activity was observed in the presence of any of the other clones (Fig. 1B, lanes 7 to 13 and 16 to 18). Thus, the region between 44.6 and 50.3 m.u. (see Fig. 1A) supplied an additional factor which was involved in TN-368 cell-specific pCAPCAT expression in the presence of the LEF library.

ORF70 is involved in late gene expression in TN-368 cells. In order to identify the gene(s) responsible for this activity, clones spanning the region overlapped by PstH5 and HC9 were tested in transient-expression assays (Fig. 2A). When a clone representing the 39.48 to 49.94 m.u. region, pXma17, was added to the LEF library, CAT activity was restored to levels comparable to those with the complete Ac*M*NPV clone library as well as those with the LEF library supplemented with HC9 (Fig. 2B, compare lane 5 with lanes 2 and 4). A clone containing the 44.68 to 49.94 m.u. region, pXA-7, was also able to restore CAT activity (Fig. 2B, compare lane 6 with lanes 2 to 4). The region of pXA-7 responsible for this activity was

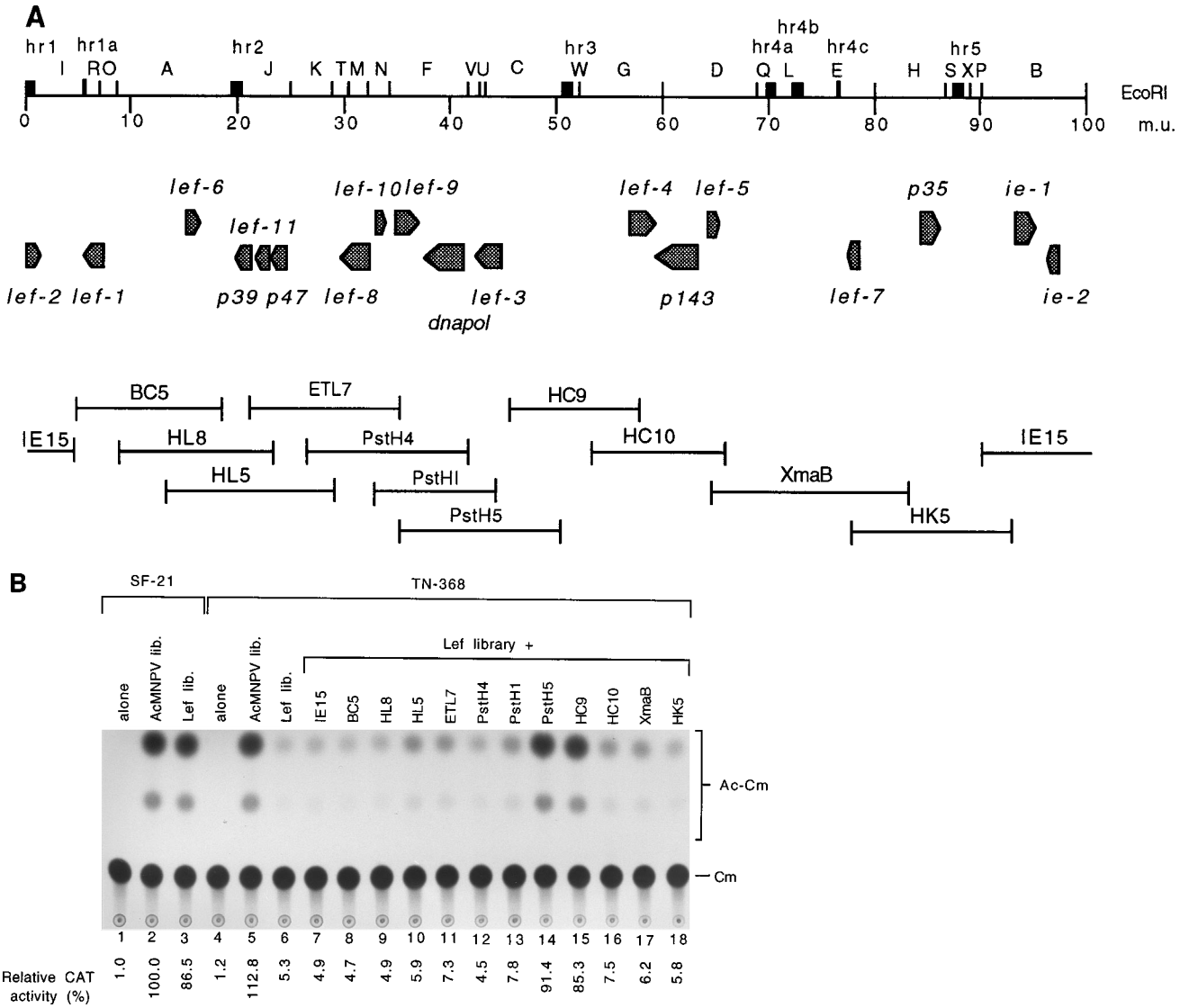

FIG. 1. Late gene expression in TN-368 cells is rescued by the 44.6 to 50.3 m.u. region of Ac*M*NPV. (A) Linear representation of the *Eco*RI restriction map of Ac*M*NPV, with the approximate locations of known LEF genes (shaded arrows, not to scale) shown relative to the overlapping Ac*M*NPV clone library. Although the exact locations of the ends of most of these Ac*M*NPV clones are not known, the ends shown represent the furthest limits of each clone. (B) The reporter plasmid pCAPCAT was transfected alone (lanes 1 and 4) or in combination with the Ac*M*NPV clone library (lanes 2 and 5) or the LEF library (lanes 3 and 6) into SF-21 and TN-368 cells, respectively. CAT expression from TN-368 cells cotransfected with pCAPCAT and the LEF library with the indicated clones of the Ac*M*NPV clone library is also shown (lanes 7 to 18). CAT activity relative to that observed with the complete Ac*M*NPV clone library in SF-21 cells (lane 2) is shown below each lane. Twofold differences are not considered significant. The positions of acetylated (Ac-Cm) and unacetylated (Cm) forms of chloramphenicol are indicated on the right. The results shown are representative of two independent experiments.

defined by using two subclones of pXA-7, pXABB4.5 and pXBS2.1. Addition of pXABB4.5 to the LEF library resulted in nearly 80-fold greater CAT activity than with the LEF library alone (Fig. 2B, compare lanes 3 and 7), while no effect on CAT expression was observed with pXBS2.1 (Fig. 2B, lane 8). A subclone of pXABB4.5, pXABgE, containing the 44.68 to 46.63 m.u. region, was able to substitute for pXABB4.5 (Fig. 2B, lane 9), whereas two other subclones from this region, pXK2.1 and pXAEBS, had no stimulatory effect on CAT levels (Fig. 2B, lanes 10 and 11, respectively).

The region between 44.68 and 46.63 m.u. contains four complete open reading frames (ORFs) identified by Ayres et al. (1) (see Fig. 2A). The functions of these ORFs during virus infection are currently unknown, although ORF71 has been found to possess significant homology to a family of genes functioning as inhibitors of apoptosis (IAPs) (2, 12). Another IAP homolog was identified previously in Ac*M*NPV but apparently is nonfunctional with respect to its ability to block actinomycin D-induced apoptosis in SF-21 cells (9). Addition of a subclone of this region (pXAIAP2) containing ORFs 71, 72, and 73 to the LEF library failed to restore CAT levels to that observed with the entire Ac*M*NPV clone library (Fig. 2B, lane 14), indicating that these three ORFs were not involved in TN-368 cell-specific pCAPCAT expression. In contrast, CAT activity was restored to Ac*M*NPV library levels when either of two clones, pXASstII1.5 or pXAMlu1.4, both containing ORF70, were added to the LEF library (Fig. 2B, lanes 12 and 13, respectively). Addition of a clone containing a frameshift mutation at the *Sst*I site within the ORF70 gene negated its stimulatory effect on pCAPCAT expression (Fig. 2B, lane 15), confirming that

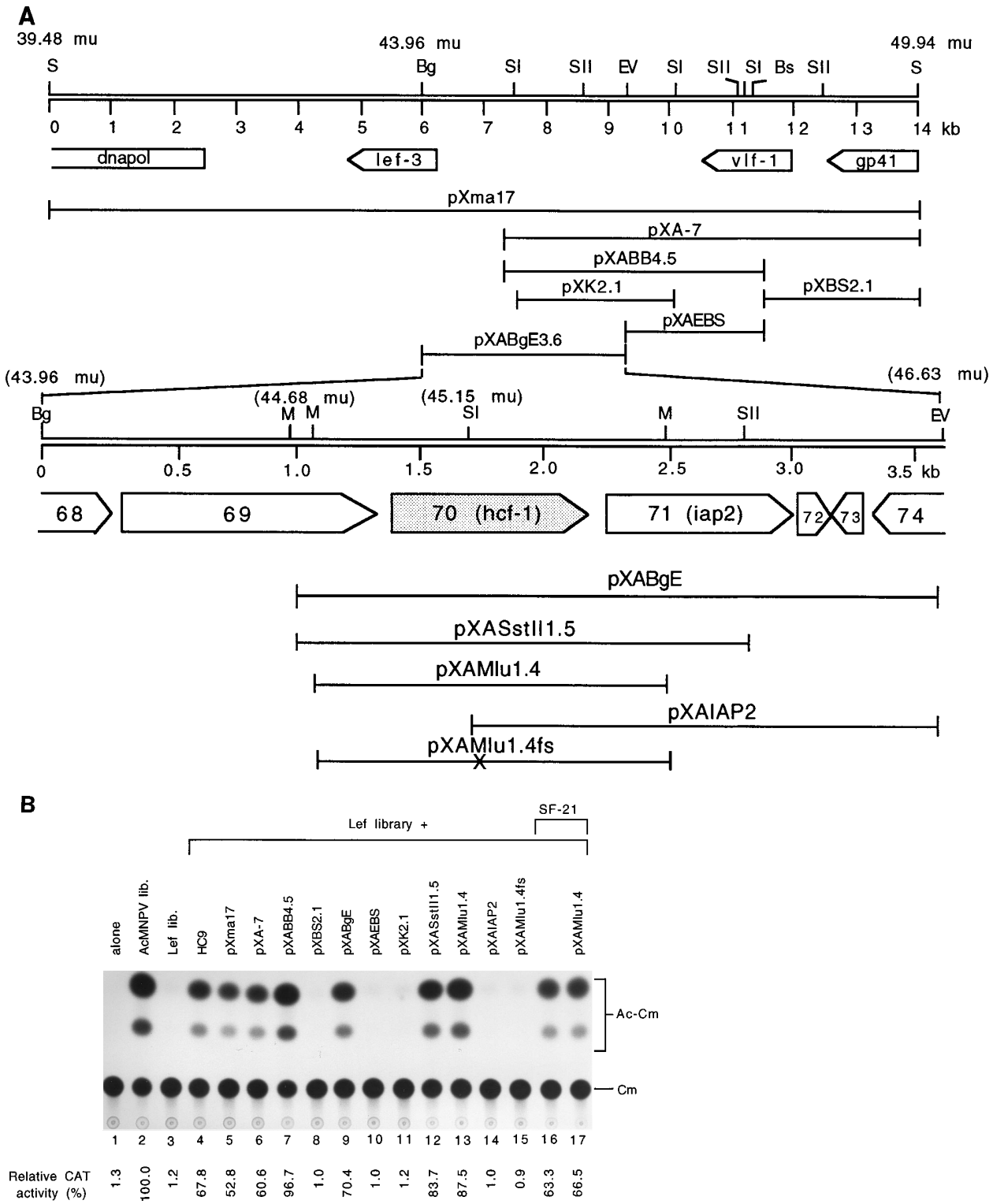

FIG. 2. ORF70 is involved in late gene expression in TN-368 but not SF-21 cells. (A) Physical map of the 39.48 to 49.94 m.u. region, showing a partial restriction map of the region along with the locations and direction of known genes. Plasmid clones used to map ORF70 (renamed *hcf-1*) as the gene required for late gene expression in TN-368 cells are indicated below. Also shown is an expanded restriction map of the 43.96 to 46.63 m.u. region, indicating the locations and orientations of ORFs as well as additional subclones used in the transient-expression assays. ORF designations and map units are according to Ayres et al. (1). Restriction enzyme sites: Bg, *Bgl*II; Bs, *Bst*EII; EV, *Eco*RV; M, *Mlu*I; S, *Sma*I; SI, *Sst*I; SII, *Sst*II. (B) pCAPCAT was transfected into TN-368 cells alone (lane 1) or in combination with the Ac*M*NPV clone library (lane 2), the LEF library (lane 3), or the LEF library with the indicated plasmid subclones from the 39.48 to 49.94 m.u. region (lanes 4 to 15). CAT expression from SF-21 cells transfected with pCAPCAT and the LEF library in the absence or presence of *hcf-1* is also shown (lanes 16 and 17). CAT activity relative to that from pCAPCAT cotransfected with the Ac*M*NPV clone library (lane 2) is shown below each lane. Twofold differences were not considered significant. The positions of acetylated (Ac-Cm) and unacetylated (Cm) chloramphenicol are indicated on the right. The results shown are representative of three independent experiments.

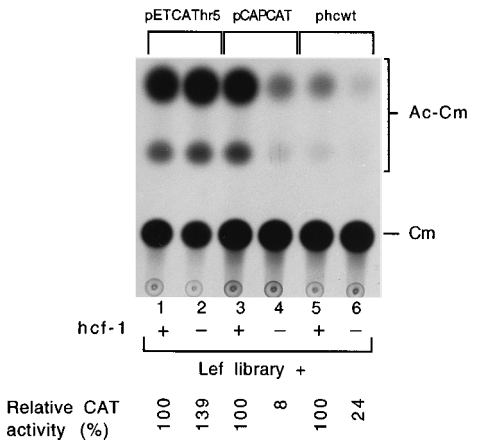

FIG. 3. Effect of *hcf-1* on early, late, and very late gene expression in TN-368 cells. The LEF library was cotransfected with reporter plasmids containing the CAT gene under early (pETCAThr5), late (pCAPCAT), and very late (phcwt) promoter control (lanes 1 and 2, 3 and 4, and 5 and 6, respectively) in the presence of *hcf-1* (+) or its frameshifted version (−). The CAT activities shown below each lane are relative to those of each of the reporter plasmids cotransfected with the LEF library and *hcf-1*. The results presented are representative of three independent experiments.

the ORF70 gene product was involved in the expression of pCAPCAT in TN-368 cells. No difference in CAT levels was observed when the LEF library was cotransfected with ORF70 in SF-21 cells (Fig. 2B, compare lanes 16 and 17), supporting the fact that ORF70 was required in TN-368 but not in SF-21 cells. We have renamed ORF70 *hcf-1*, for host cell-specific factor 1, because of its involvement in late gene expression in TN-368 but not SF-21 cells.

***hcf-1* is involved in late and very late but not early gene expression.** Since *hcf-1* apparently was involved in the expression of pCAPCAT in combination with the LEF library it was of interest to determine whether *hcf-1* had an effect on early and very late gene expression. To test this, *hcf-1* was cotransfected with the LEF library along with reporter plasmids containing the CAT gene under early (pETCAThr5) or very late (phcwt) promoter control (Fig. 3). Expression of pETCAThr5 was unaffected by replacement of *hcf-1* by its frameshifted version (Fig. 3, compare lanes 1 and 2). In contrast, substitution of the frameshifted *hcf-1* resulted in dramatic decreases in CAT expression from both pCAPCAT and phcwt (Fig. 3, compare lanes 3 and 4 and lanes 5 and 6, respectively), indicating the involvement of a functional *hcf-1* gene product in late and very late gene expression. Thus, *hcf-1* can also be considered a LEF gene required for optimal late and very late gene expression in TN-368 cells. The approximately 20-fold difference in CAT expression levels observed between pCAPCAT and phcwt in the presence of the complete LEF library (including *hcf-1*) (Fig. 3, compare lanes 3 and 5) suggests that additional very late gene expression factors may be required for optimal expression from the *polh* promoter in TN-368 cells; a similar difference is also observed in SF-21 cells.

***ie-2*, *lef-7*, and *p35* are not required for late gene expression in TN-368 cells.** Since there was a differential involvement of *hcf-1* in late and very late gene expression between TN-368 and SF-21 cells, we tested whether there was also a differential involvement of the other LEFs in late gene expression between the two cell lines. This was done by determining the effect of omitting one LEF at a time from the LEF library, including *hcf-1*, on pCAPCAT expression (Fig. 4). In the presence of the complete LEF library and *hcf-1*, the levels of CAT expression in both cell lines were comparable (compare lanes 2 in Fig. 4A and B). Removal of each LEF individually from the library resulted in a 20- to 70-fold reduction in CAT activity in SF-21 cells (Fig. 4A, compare lane 2 and lanes 3 to 15 and 17 to 21). As expected, removal of *hcf-1* from the library had no effect on pCAPCAT expression (Fig. 4A, lane 16) in SF-21 cells. In comparison, removal of three of the LEFs, *ie-2*, *lef-7*, and *p35*, had very little effect on CAT levels in TN-368 cells (Fig. 4B, compare lane 2 with lanes 4, 20, and 21), indicating that these LEFs were differentially required for late gene expression between the two cell lines. Omission of each of the other LEFs from the library (except *hcf-1*) reduced CAT levels at least 10- to 50-fold in TN-368 cells (Fig. 4B, compare lane 3 with lanes 5 to 19).

***hcf-1* is involved in reporter plasmid DNA replication or stability.** The role that *hcf-1* played in late gene expression in TN-368 cells was further defined by determining whether *hcf-1* was involved in the replication of the reporter plasmid pCAPCAT. The dependence of pCAPCAT expression on replication of the reporter plasmid was previously demonstrated in a transient-replication assay in which 9 of the 18 LEFs were shown to be directly or indirectly involved in plasmid DNA replication in SF-21 cells (35). A similar assay based on the resistance of replicated plasmid DNA to cleavage by *Dpn*I was used to test the involvement of *hcf-1* and the nine replication-related LEFs in pCAPCAT DNA replication or stability in TN-368 cells (Fig. 5).

TN-368 cells were cotransfected with pCAPCAT and the nine replication-related LEFs, *ie-1*, *ie-2*, *lef-1*, *lef-2*, *lef-3*, *dnapol*, *p143*, *lef-7*, and *p35* (known as the LEF replication library). The reporter plasmid pCAPCAT contains *hr5*, a *cis*-acting sequence which serves as an Ac*M*NPV-activated origin of DNA replication in transient plasmid replication assays (30, 35, 52). Total intracellular DNA was isolated at 96 h posttransfection and digested with *Bgl*II to linearize the plasmid DNA (7.2 kb) and with *Dpn*I to distinguish between input and replicated plasmid DNAs. When pCAPCAT was cotransfected with the LEF replication library, no plasmid DNA replication was observed (Fig. 5, lane 1); however, addition of *hcf-1* to the LEF replication library stimulated pCAPCAT plasmid DNA replication about 70-fold (Fig. 5, compare lanes 1 and 2), indicating that HCF-1 affected the level of plasmid DNA replication or stability. Omission of *ie-1*, *lef-1*, *lef-2*, *lef-3*, and *p143* from the LEF replication library dramatically reduced levels of pCAPCAT replication, approximately 20- to 70-fold (Fig. 5, compare lane 2 with lanes 3, 5 to 7, and 9), while omission of *ie-2*, *dnapol*, *lef-7*, and *p35* resulted in differences of less than 2.5-fold (Fig. 5, lanes 4, 8, 10, and 11, respectively). Thus, it appears that while *ie-1*, *lef-1*, *lef-2*, *lef-3*, *p143*, and *hcf-1* are necessary for plasmid DNA replication or stability in TN-368 cells, *ie-2*, *dnapol*, *lef-7*, and *p35* have little or no effect on plasmid DNA replication or stability in this transient-replication assay system. Omission of *dnapol* from the LEF replication library also had a minor effect on plasmid replication in SF-21 cells (35), suggesting that a host DNA polymerase can substitute for this function, at least in transient-replication assays.

DISCUSSION

We have observed several differences in the requirement for LEF genes in transient-expression assays between the SF-21 and TN-368 lepidopteran cell lines, both of which are fully permissive for Ac*M*NPV infection. Whereas *ie-2*, *lef-7*, and *p35* were required for optimal late gene expression as part of a library of 18 LEF genes in SF-21 cells, they did not contribute

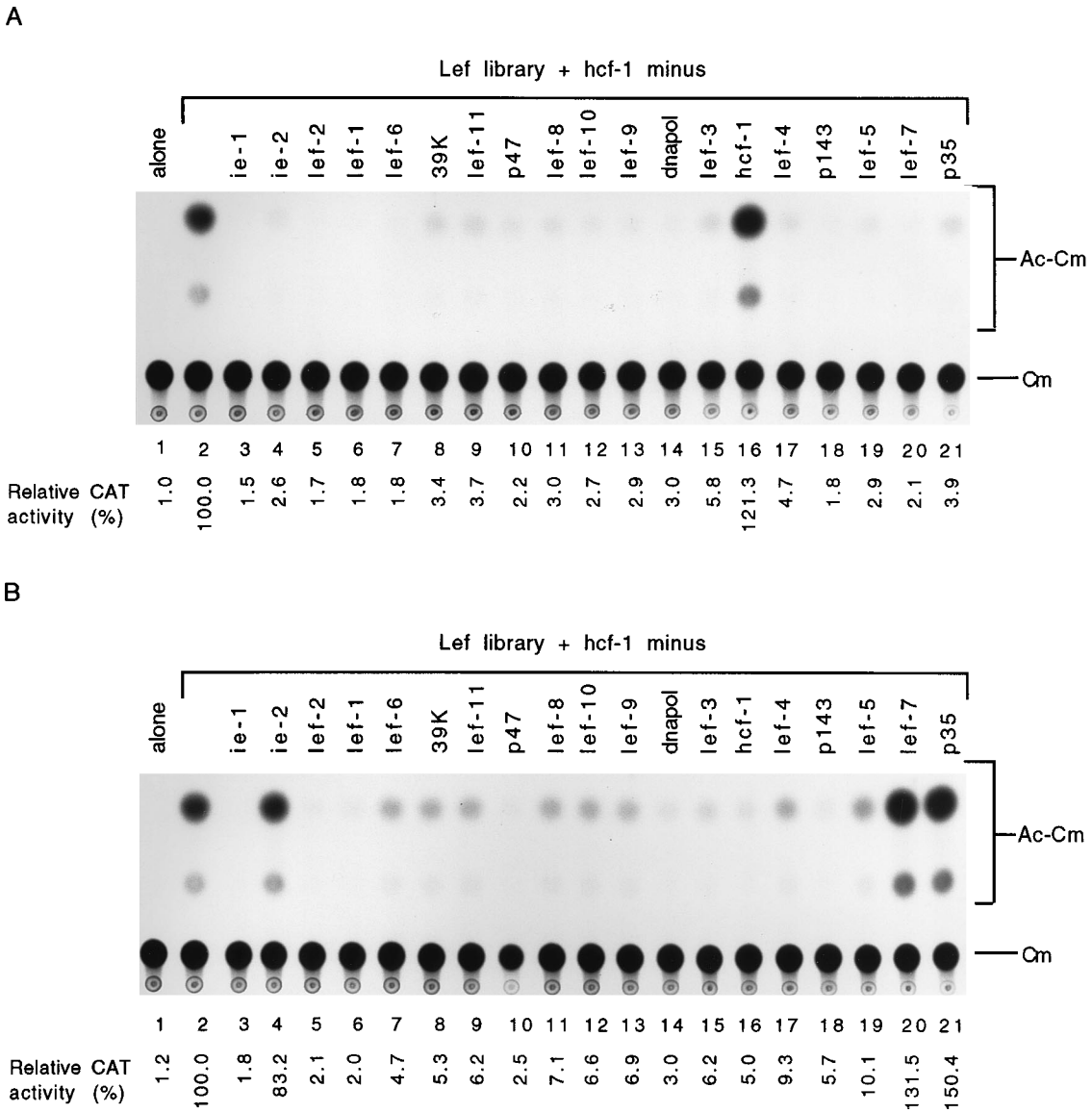

FIG. 4. Differences in LEF requirements between the SF-21 and TN-368 cell lines. The reporter plasmid pCAPCAT was transfected into SF-21 (A) or TN-368 (B) cells either alone (lane 1) or in combination with *hcf-1* and the LEF library with one LEF omitted at a time (lanes 3 to 21). The LEF omitted from each transfection is indicated above each lane. CAT activities relative to that in cells cotransfected with pCAPCAT and *hcf-1* and the LEF library are indicated below each lane. The results presented are representative of two independent experiments.

significantly to transient late gene expression in TN-368 cells. Conversely, a novel baculovirus gene, *hcf-1*, was required for optimal transient late gene expression in TN-368 cells but not SF-21 cells. Previous studies have demonstrated a differential requirement for *p35* in virus replication in SF-21 but not TN-368 cells (7, 8, 10, 23), and this requirement extends to the organism level as well. There is also evidence of cell-specific differences in the level of *ie-2*-mediated transactivation of the *ie-1* promoter (58). Whether the differential requirements for *ie-2*, *lef-7*, and *hcf-1* also extend to virus infections at the cellular and organismal levels remains to be established.

Like *ie-2*, *lef-7*, and *p35*, the involvement of *hcf-1* in transient late expression was correlated with its contribution to reporter plasmid DNA replication or stability. We are not certain whether *hcf-1* contributed to the expression of the "replicative LEFs" (e.g., *lef-1* and *dnapol*) or contributed directly to reporter DNA replication or stability. The former possibility seems less likely, since *hcf-1* did not strongly affect expression from the early *etl* promoter (Fig. 3), nor did it significantly influence expression from the *lef-3* and *lef-7* promoters (36). *hcf-1* did not functionally substitute for either *lef-7* or *ie-2* in SF-21 cells, and therefore, *hcf-1* possesses functions distinct from those of these two LEFs. No extensive homologies to other genes within the nucleic acid or protein databases were found for *hcf-1*, which is predicted to encode a very cysteine-rich (10%) polypeptide with several motifs suggestive of metal ion binding. Like *p35*, *hcf-1* may contribute to plasmid DNA stability rather than DNA replication per se. However, *hcf-1* does not appear to function to block apoptosis because (i) neither *p35*, a gene which functions to block apoptosis in a very broad range of species, nor the antiapoptotic IAPs functionally substitute for *hcf-1* (36) and (ii) we have been unable to detect

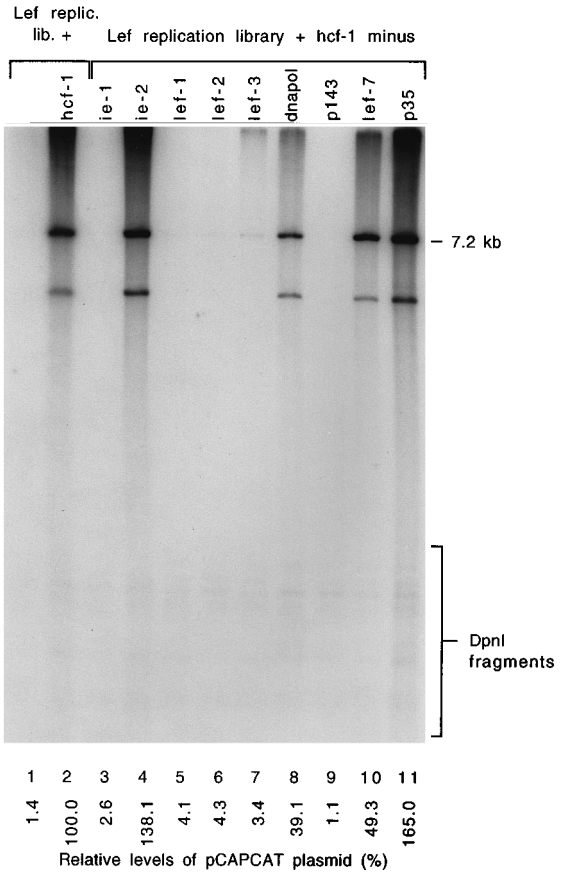

FIG. 5. Contributions of LEFs to reporter plasmid replication in TN-368 cells. pCAPCAT was cotransfected with the LEF replication library (lane 1), the LEF replication library and *hcf-1* (lane 2), or the LEF replication library and *hcf-1* with each replication-related LEF (shown above each lane) omitted (lanes 3 to 11). Total intracellular DNA was prepared from transfected cells at 96 h posttransfection and digested with *Bgl*II and *Dpn*I. The DNA was transferred to Zeta Probe membranes and hybridized to radiolabeled pCAPCAT plasmid DNA. The position of the linearized *Dpn*I-resistant pCAPCAT DNA (7.2 kb) is indicated by an arrow. The additional band also present in lanes containing replicated pCAPCAT DNA represents a coreplicating LEF plasmid. Levels of replicated plasmid recovered from each transfection were quantitated relative to the level observed in cells transfected with *hcf-1* and the LEF replication library (lane 2) and are shown below each lane. The results presented are representative of two independent experiments.

apoptosis in TN-368 cells transfected with the LEF library lacking *hcf-1*. Thus, a more precise role for *hcf-1* remains to be determined.

The fact that five "replication-associated" LEFs (*ie-2*, *lef-7*, *p35*, *hcf-1*, and *p143*) have been implicated in baculovirus host range determination through these studies and others (8, 11, 23, 37) suggests that the ability of baculoviruses to activate and maintain viral DNA replication may be more critical in viral host range determination than the nature of the late transcriptional apparatus, which is probably specified to a large extent by the products of the "transcription-specific" LEFs. However, additional restrictions to productive infections may also occur after the initiation of viral DNA replication (21a, 40).

It will be important to determine whether the differential contribution of *ie-2*, *lef-7*, and *hcf-1* observed in transient-expression assays extends to the context of a viral infection. We have generated an *hcf-1* knockout mutant which replicates normally in SF-21 cells but is severely compromised in its ability to replicate in TN-368 cells. We are currently studying the properties of this mutant in vivo and in cell culture to better understand the role of HCF-1 during virus infection at both the cellular and organismal levels.

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REFERENCES

1. **Ayres, M. D., S. C. Howard, J. Kuzio, M. Lopez-Ferber, and R. D. Possee.** 1994. The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. Virology **202:**586–605.
2. **Birnbaum, M. J., R. J. Clem, and L. K. Miller.** 1994. An apoptosis-inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. J. Virol. **68:**2521–2528.
3. **Blissard, G. W., and G. F. Rohrmann.** 1990. Baculovirus diversity and biology. Annu. Rev. Entomol. **35:**127–155.
4. **Carbonell, L. F., M. J. Klowden, and L. K. Miller.** 1985. Baculovirus-mediated expression of bacterial genes in dipteran and mammalian cells. J. Virol. **56:**153–160.
5. **Carson, D. D., M. D. Summers, and L. A. Guarino.** 1991. Molecular analysis of a baculovirus regulatory gene. Virology **182:**279–286.
6. **Carson, D. D., M. D. Summers, and L. A. Guarino.** 1991. Transient expression of *Autographa californica* nuclear polyhedrosis virus immediate-early gene IE-N is regulated by three viral elements. J. Virol. **65:**945–951.
7. **Clem, R. J., M. Fechheimer, and L. K. Miller.** 1991. Prevention of apoptosis by a baculovirus gene during infection of insect cells. Science **254:**1388–1390.
8. **Clem, R. J., and L. K. Miller.** 1993. Apoptosis reduces both the in vitro replication and in vivo infectivity of a baculovirus. J. Virol. **67:**3730–3738.
9. **Clem, R. J., and L. K. Miller.** 1994. Control of programmed cell death by the baculovirus genes *p35* and *iap*. Mol. Cell. Biol. **14:**5212–5222.
10. **Clem, R. J., and L. K. Miller.** 1994. Induction and inhibition of apoptosis by insect viruses, p. 89–110. *In* L. D. Tomei (ed.), Apoptosis II: the molecular basis of cell death. Cold Spring Harbor Laboratory Press, Plainview, N.Y.
11. **Croizier, G., L. Croizier, O. Argaud, and D. Poudevigne.** 1994. Extension of *Autographa californica* nuclear polyhedrosis virus host range by interspecific replacement of a short DNA sequence in the p143 helicase gene. Proc. Natl. Acad. Sci. USA **91:**48–52.
12. **Crook, N. E., R. J. Clem, and L. K. Miller.** 1993. An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. J. Virol. **67:**2168–2174.
13. **Erlandson, M. A., J. Gordon, and E. B. Carstens.** 1985. Size and map locations of early transcription products on the *Autographa californica* nuclear polyhedrosis virus genome. Virology **142:**12–23.
14. **Fuchs, L. Y., M. S. Woods, and R. F. Weaver.** 1983. Viral transcription during *Autographa californica* nuclear polyhedrosis virus infection: a novel RNA polymerase induced in infected *Spodoptera frugiperda* cells. J. Virol. **43:**641–646.
15. **Glocker, B., R. R. Hoopes, Jr., L. Hodges, and G. F. Rohrmann.** 1993. In vitro transcription from baculovirus late gene promoters: accurate mRNA initiation by nuclear extracts prepared from infected *Spodoptera frugiperda* cells. J. Virol. **67:**3771–3776.
16. **Gordon, J. D., and E. B. Carstens.** 1984. Phenotypic characterization and physical mapping of a temperature-sensitive mutant of *Autographa californica* nuclear polyhedrosis virus defective in DNA synthesis. Virology **138:** 69–81.
17. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:**1044–1051.
18. **Grula, M. A., P. L. Buller, and R. F. Weaver.** 1981. Alpha-amanitin-resistant viral RNA synthesis in nuclei isolated from nuclear polyhedrosis virus-infected *Heliothis zea* larvae and *Spodoptera frugiperda* cells. J. Virol. **38:**916–921.
19. **Guarino, L. A., and W. Dong.** 1991. Expression of an enhancer-binding protein in insect cells transfected with the *Autographa californica* nuclear polyhedrosis virus IE1 gene. J. Virol. **65:**3676–3680.
20. **Guarino, L. A., and M. D. Summers.** 1986. Functional mapping of a *trans*-activating gene required for expression of a baculovirus delayed-early gene. J. Virol. **57:**565–571.
21. **Guarino, L. A., and M. D. Summers.** 1987. Nucleotide sequence of a baculovirus regulatory gene. J. Virol. **61:**2091–2099.

21a. **Guzo, D., H. Rathburn, K. Guthrie, and E. Dougherty.** 1992. Viral and host cellular transcription in *Autographa californica* nuclear polyhedrosis virus-infected gypsy moth cell lines. J. Virol. **66:**2966–2972.

22. **Hay, B. A., T. Wolff, and G. M. Rubin.** 1994. Expression of baculovirus P35 prevents cell death in *Drosophila*. Development **120:**2121–2129.

23. **Hershberger, P. A., J. A. Dickson, and P. D. Friesen.** 1992. Site-specific mutagenesis of the 35-kilodalton protein gene encoded by *Autographa californica* nuclear polyhedrosis virus: cell line-specific effects on virus replication. J. Virol. **66:**5525–5533.
24. **Hink, W. F.** 1970. Established insect cell line from the cabbage looper *Trichoplusia ni*. Nature (London) **226:**466–467.
25. **Hoopes, R. R., and G. F. Rohrmann.** 1991. *In vitro* transcription of baculovirus immediate early genes: accurate messenger RNA initiation by nuclear extracts from both insect and human cells. Proc. Natl. Acad. Sci. USA **88:**4513–4517.
26. **Huh, N. E., and R. F. Weaver.** 1990. Identifying the RNA polymerases that synthesize specific transcripts of *Autographa californica* nuclear polyhedrosis virus. J. Gen. Virol. **71:**195–202.
27. **Kool, M., C. H. Ahrens, R. W. Goldbach, and G. F. Rohrmann.** 1994. Identification of genes involved in DNA replication of the *Autographa californica* baculovirus. Proc. Natl. Acad. Sci. USA **91:**11212–11216.
28. **Kovacs, G. R., L. A. Guarino, and M. D. Summers.** 1991. Novel regulatory properties of the IE1 and IE0 transactivators encoded by the baculovirus *Autographa californica* multicapsid nuclear polyhedrosis virus. J. Virol. **65:** 5281–5288.
29. **Krappa, R., and D. Knebel-Morsdorf.** 1991. Identification of the very early transcribed baculovirus gene PE-38. J. Virol. **65:**805–812.
30. **Leisy, D. J., and G. F. Rohrmann.** 1993. Characterization of the replication of plasmids containing *hr* sequences in baculovirus-infected *Spodoptera frugiperda* cells. Virology **196:**722–730.
31. **Li, Y., A. L. Passarelli, and L. K. Miller.** 1993. Identification, sequence, and transcriptional mapping of *lef-3*, a baculovirus gene involved in late and very late gene expression. J. Virol. **67:**5260–5268.
32. **Lu, A., and E. B. Carstens.** 1991. Nucleotide sequence of a gene essential for viral DNA replication in the baculovirus *Autographa californica* nuclear polyhedrosis virus. Virology **181:**336–347.
33. **Lu, A., and E. B. Carstens.** 1993. Immediate-early baculovirus genes transactivate the p143 gene promoter of *Autographa californica* nuclear polyhedrosis virus. Virology **195:**710–718.
34. **Lu, A., and L. K. Miller.** 1994. Identification of three late expression factor genes within the 33.8- to 43.4-map-unit region of *Autographa californica* nuclear polyhedrosis virus. J. Virol. **68:**6710–6718.
35. **Lu, A., and L. K. Miller.** 1995. The roles of eighteen late expression factor genes in transcription and DNA replication. J. Virol. **69:**975–982.
36. **Lu, A., and L. K. Miller.** Unpublished results.
37. **Maeda, S., S. G. Kamita, and A. Kondo.** 1993. Host range expansion of *Autographa californica* nuclear polyhedrosis virus (NPV) following recombination of a 0.6-kilobase-pair DNA fragment originating from *Bombyx mori* NPV. J. Virol. **67:**6234–6238.
38. **McLachlin, J. M., and L. K. Miller.** 1994. Identification and characterization of *vlf-1*, a baculovirus gene involved in very late gene expression. J. Virol. **68:**7746–7756.
39. **Morris, T. D., and L. K. Miller.** 1992. Promoter influence on baculovirus-mediated gene expression in permissive and nonpermissive insect cell lines. J. Virol. **66:**7397–7405.
40. **Morris, T. D., and L. K. Miller.** 1993. Characterization of productive and non-productive AcMNPV infection in selected insect cell lines. Virology **197:**339–348.
41. **Morris, T. D., J. W. Todd, B. Fisher, and L. K. Miller.** 1994. Identification of *lef-7*, a baculovirus gene affecting late gene expression. Virology **200:**360–369.
42. **Nissen, M. S., and P. D. Friesen.** 1989. Molecular analysis of the transcriptional regulatory region of an early baculovirus gene. J. Virol. **63:**493–503.
43. **Ooi, B. G., and L. K. Miller.** 1988. Regulation of host RNA levels during baculovirus infection. Virology **166:**515–523.
44. **O'Reilly, D. R., A. M. Crawford, and L. K. Miller.** 1989. Viral proliferating cell nuclear antigen. Nature (London) **337:**606.
45. **O'Reilly, D. R., L. K. Miller, and V. A. Luckow.** 1992. Baculovirus expression vectors: a laboratory manual. W. H. Freeman and Co., New York.
46. **O'Reilly, D. R., A. L. Passarelli, I. F. Goldman, and L. K. Miller.** 1990. Characterization of the DA26 gene in a hypervariable region of the *Autographa californica* nuclear polyhedrosis virus genome. J. Gen. Virol. **71:**2149–2158.
47. **Passarelli, A. L., and L. K. Miller.** 1993. Three baculovirus genes involved in late and very late gene expression: *ie-1*, *ie-n*, and *lef-2*. J. Virol. **67:**2149–2158.
48. **Passarelli, A. L., and L. K. Miller.** 1993. Identification and characterization of *lef-1*, a baculovirus gene involved in late and very late gene expression. J. Virol. **67:**3281–3488.
49. **Passarelli, A. L., and L. K. Miller.** 1993. Identification of genes encoding late expression factors located between 56.0 and 65.4 map units of the *Autographa californica* nuclear polyhedrosis virus genome. Virology **197:**704–714.
50. **Passarelli, A. L., and L. K. Miller.** 1994. Identification and transcriptional regulation of the baculovirus *lef-6* gene. J. Virol. **68:**4458–4467.
51. **Passarelli, A. L., J. W. Todd, and L. K. Miller.** 1994. A baculovirus gene involved in late gene expression encodes a large polypeptide with a conserved motif of RNA polymerases. J. Virol. **68:**4673–4678.
52. **Pearson, M. N., R. M. Bjornson, G. Pearson, and G. F. Rohrmann.** 1992. The *Autographa californica* baculovirus genome: evidence for multiple replication origins. Science **257:**1382–1384.
53. **Rabizadeh, S., D. J. Lacount, P. D. Friesen, and D. E. Bredesen.** 1993. Expression of the baculovirus p35 gene inhibits mammalian neural cell death. J. Neurochem. **61:**2318–2321.
54. **Rankin, C., B. G. Ooi, and L. K. Miller.** 1988. Eight base pairs encompassing the transcriptional start point are the major determinant for baculovirus polyhedrin gene expression. Gene **70:**39–50.
55. **Ribeiro, B., K. Hutchinson, and L. K. Miller.** 1994. A mutant baculovirus with a temperature-sensitive IE-1 transregulatory protein. J. Virol. **68:**1075–1084.
56. **Rice, W. C., and L. K. Miller.** 1986. Baculovirus transcription in the presence of inhibitors and in nonpermissive *Drosophila* cells. Virus Res. **6:**155–172.
57. **Sugimoto, A., P. D. Friesen, and J. H. Rothman.** 1994. Baculovirus p35 prevents developmentally programmed cell death and rescues a ced-9 mutant in the nematode *Caenorhabditis elegans*. EMBO J. **13:**2023–2028.
58. **Thielmann, D. A., and S. Stewart.** 1992. Molecular analysis of the *trans*-activating IE-2 gene of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology **187:**84–96.
59. **Thiem, S. M., and L. K. Miller.** 1990. Differential gene expression mediated by late, very late, and hybrid baculovirus promoters. Gene **91:**87–94.
60. **Todd, J. W., A. L. Passarelli, and L. K. Miller.** 1995. Eighteen baculovirus genes, including *lef-11*, *p35*, *39K*, and *p47*, support late gene expression. J. Virol. **69:**968–974.
61. **Todd, J. W., A. L. Passarelli, and L. K. Miller.** Submitted for publication.
62. **Tomalski, M. D., J. Wu, and L. K. Miller.** 1988. The location, sequence, transcription and regulation of a baculovirus DNA polymerase gene. Virology **167:**591–600.
63. **Vaughn, J. L., R. H. Goodwin, G. J. Tompkins, and P. McCawley.** 1977. The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera: Noctuidae). In Vitro **13:**213–217.
64. **Yang, C. L., D. A. Stetler, and R. F. Weaver.** 1991. Structural comparison of the *Autographa californica* nuclear polyhedrosis virus-induced RNA polymerase and the three nuclear RNA polymerases from the host, *Spodoptera frugiperda*. Virus Res. **20:**251–264.
65. **Yoo, S., and L. M. Guarino.** 1994. The *Autographa californica* nuclear polyhedrosis virus *ie-2* gene encodes a transcriptional regulator. Virology **202:** 746–753.